Supporting information for

Bacterial-host adhesion dominated by collagen subtypes remodeled by osmotic pressures

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Other Supplementary Materials for this manuscript include the following:

Movie S1

Data S1

Supplementary Text

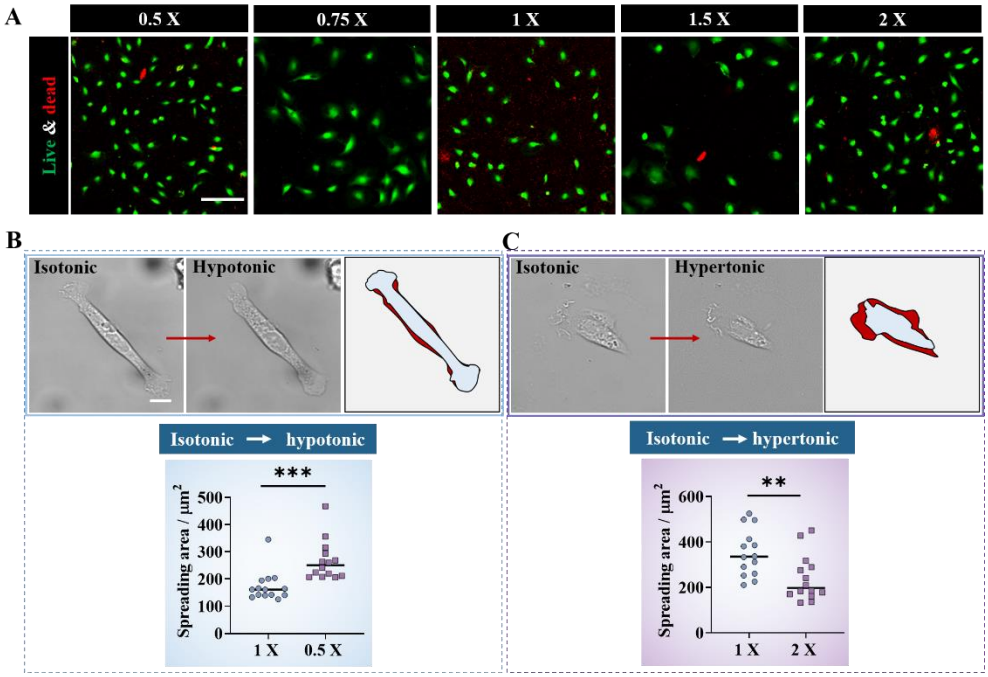

Fig. S1. Cytotoxicity assay and morphological changes under osmotic stresses. (A) Results of live/dead staining after IEC-6 cells were exposed to hypotonic (0.5 X and 0.75 X), isotonic (1 X), and hypertonic (1.5 X and 2 X) solutions for 3 h, where green denoted the live cells whereas red indicated the dead ones. Scale bar: 100 μm. (B) and (C) changes in cell morphology after 3 min of hypotonic and hypertonic stimulation. The spreading areas of the cells were quantified via the software of ImageJ. Scale bar: 10 μm. At least three independent experiments were carried out for each condition. Two-sided unpaired t test was used for statistical analysis. **and*** indicated $P < 0.01$ and $P < 0.001$, respectively.

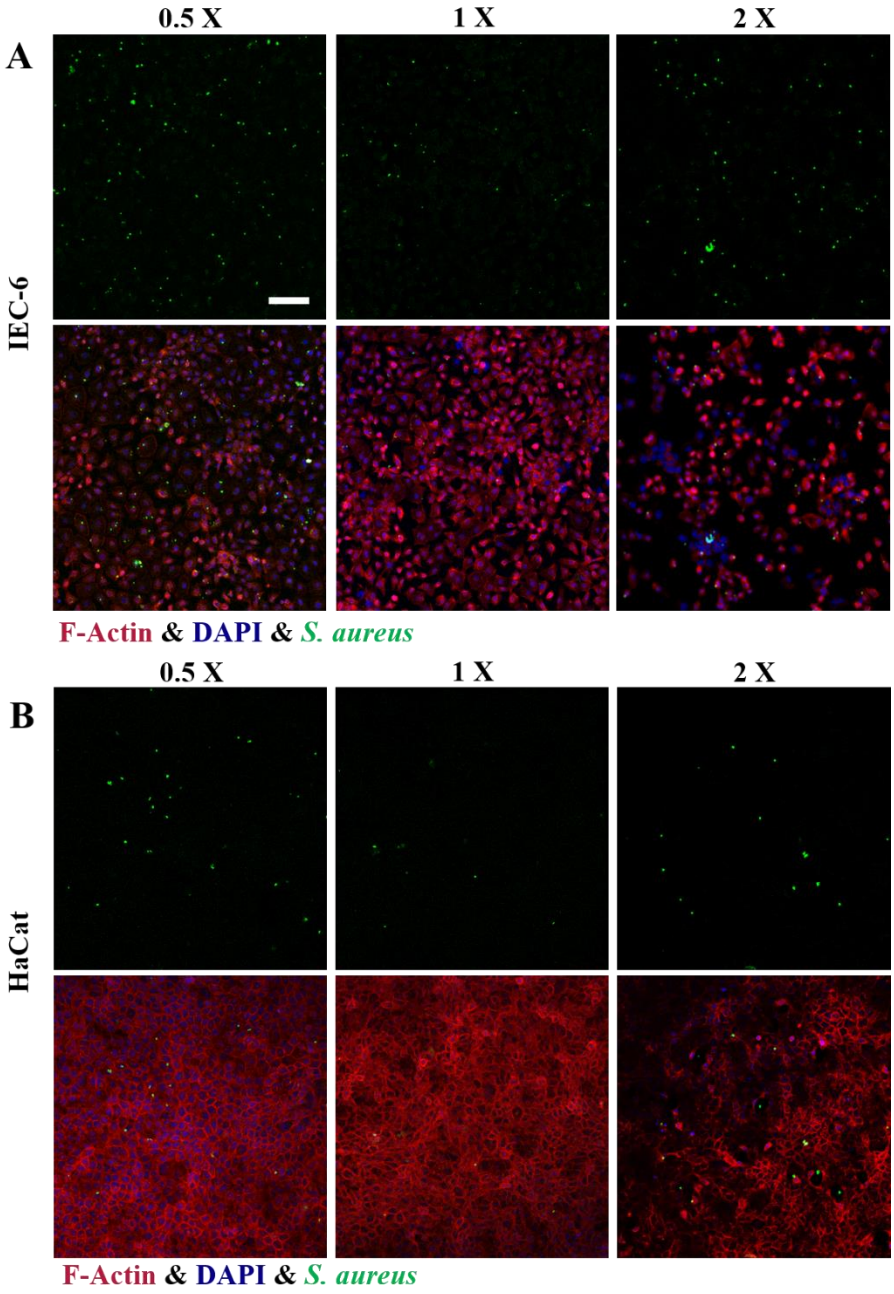

Fig. S2. Interactions between Staphylococcus aureus (*S. aureus*) expressing green fluorescent protein (GFP) and host cell monolayers of IEC-6 (A) or HaCat cell (B). Scale bar: 50 μm.

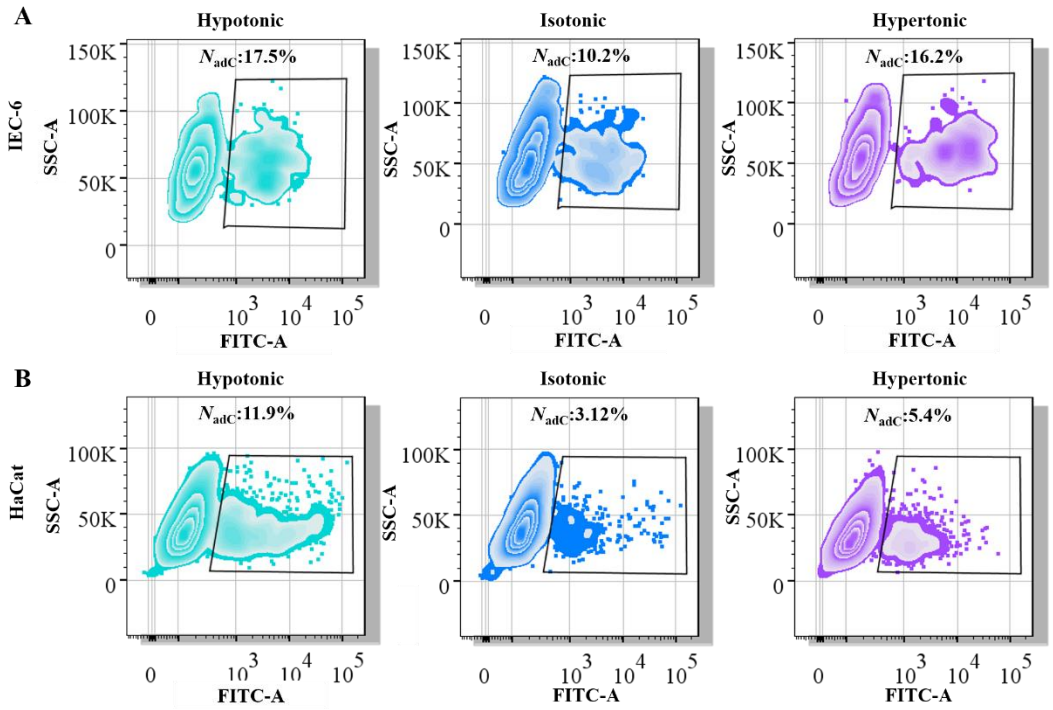

Fig. S3. Interactions between bacteria (*S. aureus*) and host cells including (A) IEC-6 cells and (B) HaCat cells, regulated with environmental osmotic pressures, where the host cells carrying bacteria, *i.e.*, the host cells to which bacteria adhered and internalized, were quantified through the flow cytometry. The percentages of the host cells carrying bacteria were also presented in the images.

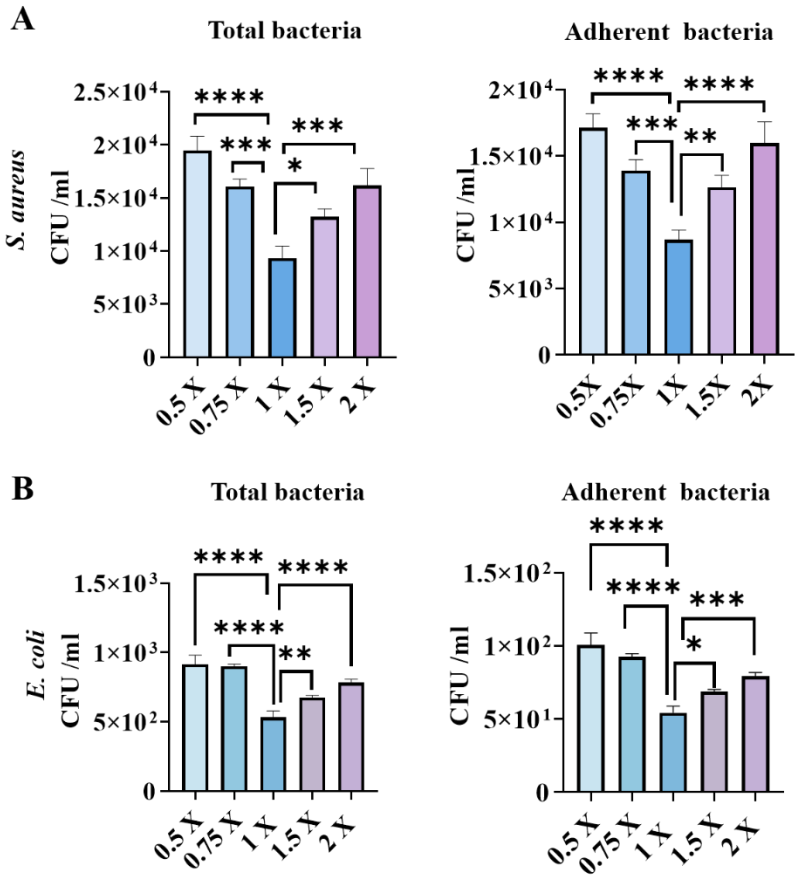

Fig. S4. Colony counts of bacteria that directly interacted with the host cells, including adherent bacteria and internalized bacteria (hereafter referred to as total bacteria) and purely adherent bacteria. (A) and (B) were the corresponding experimental results, in which the adopted bacteria were gram-positive bacterium *S. aureus* and gram-negative bacterium *E. coli*, respectively. In these experiments, the host cells were allowed to interact with GFP-expressing *S. aureus*. Then, they were rinsed three times with Dulbecco's phosphate buffered saline (DPBS) solution. To quantify the number of total bacteria that included adherent bacteria and internalized bacteria, the host cells harboring adherent and internalized bacteria were lysed with 1% Triton X-100 and subsequently serially diluted for analysis based on the flat colony counting method. On the other hand, the host cells were treated with 200 μg/mL gentamicin to kill extracellularly adherent bacteria. In this way, one could determine the number of internalized bacteria. Further, the cells were lysed with 1% Triton X-100 and serially diluted for analysis using the same flat colony counting method. Finally, the purely adherent bacteria might be quantitatively estimated. At least three independent

experiments were carried out for each specific experimental condition. All the statistical data were denoted as Mean±SD, and one-way analysis of variance (ANOVA) was employed in the data analyses with ** and *** indicating $P < 0.01$ and $P < 0.001$, respectively.

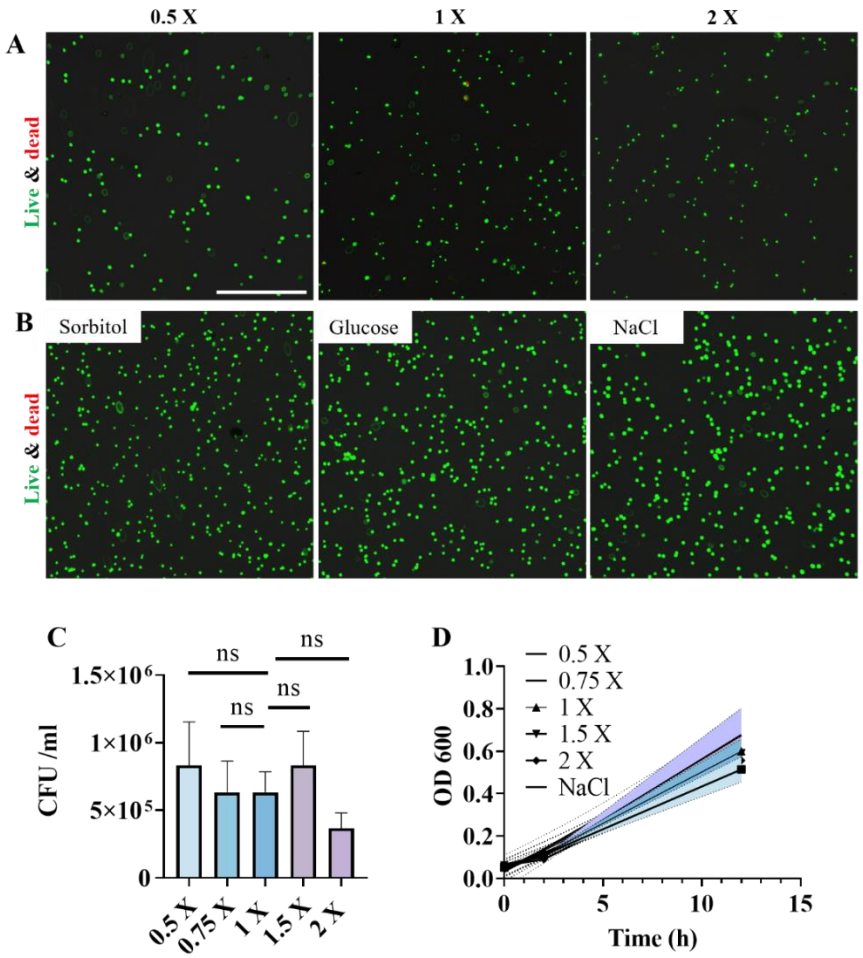

Fig. S5. Bacterial viability tests under different osmotic pressures. (A) Fluorescence images presenting the viability of *S. aureus*, where the live and dead bacteria were labelled as green and red by SYTO/PI dead-live double staining, respectively, after they were treated with hypotonic (0.5 X, left), isotonic (1 X, middle) and hypertonic (2 X, right) solutions for 6 h. (B) Typical fluorescence images presenting the viability of *S. aureus* after they were treated with the hypertonic solutions prepared with sorbitol (left), glucose (middle) and NaCl (right), respectively. (C) Colony count-based statistical results of the bacteria treated with hypotonic (0.5 X, 0.75 X), isotonic (1 X) and hypertonic (1.5 X, 2 X) solutions for 6 h, respectively. (D) Growth curves (OD 600) of the bacteria in the hypotonic (0.5 X and 0.75 X), isotonic (1 X) and hypertonic (1.5 X and 2 X) solutions. All the statistical data were presented as Mean±SD from at least three independent experiments for each specific condition. Statistical analyses based on one-way ANOVA were used in the experiments and *, **, *** and **** denoted $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively. Scale bar: 100 μm.

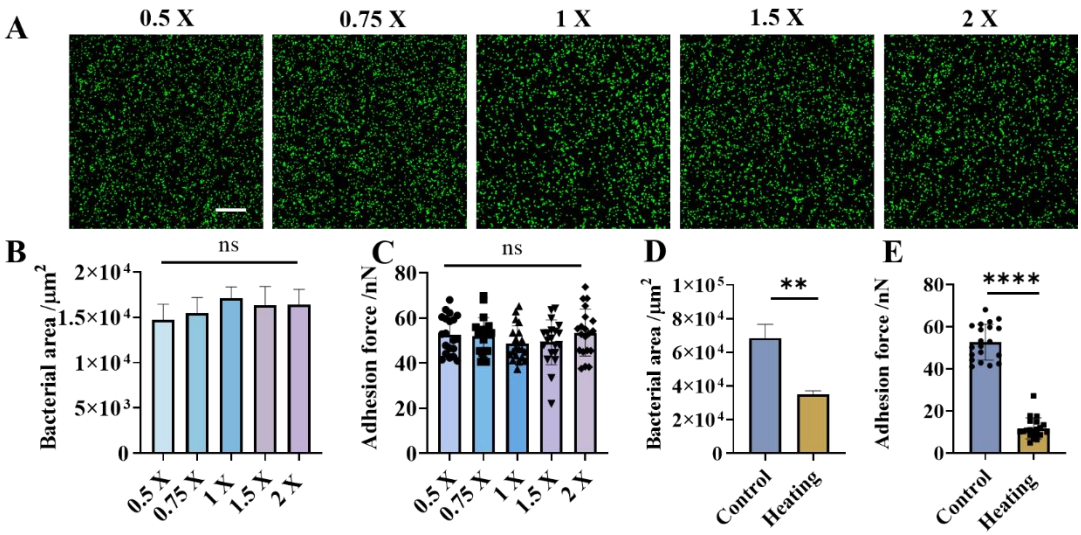

Fig. S6. Experimental results of bacterial adhesion to collagen-modified substrates under different osmotic pressures. In these experiments, bacteria (*S. aureus*) were added to collagen-modified substrates and then treated with the hypotonic (0.5 X and 0.75 X), isotonic (1 X) and hypertonic (1.5 X and 2 X) solutions for 6 h, respectively. Subsequently, they were rinsed three times to remove the nonadherent bacteria and imaged with an inverted confocal laser scanning microscope (Nikon A1, Japan). (A) Typical Fluorescence images of bacterial adhesion to collagen-modified substrates under different osmotic pressures. (B) Bacterial adhesion areas under different osmotic pressures. (C) Adhesion forces between the bacteria and the underlying collagen-modified substrates under different osmotic pressures. (D) Comparison of bacterial adhesion area between unheated and heated treatments. (E) Comparison of adhesion force between unheated and heated treatments. All these statistical results were presented as Mean ±SD from at least three independent experiments for each specific condition. Statistical analyses based on one-way ANOVA were utilized in the experiments and *, **, ***and **** denoted $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively. Scale bar 100 μm.

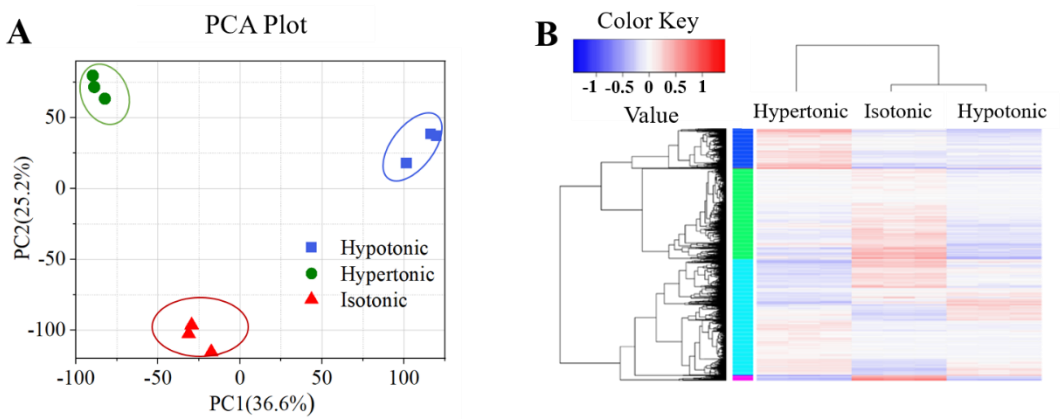

Fig. S7. RNA sequencing analysis were performed on host cells (IEC-6) treated with the hypotonic (0.5 X), isotonic (1 X) and hypertonic (2 X) solutions, respectively. (A) Principal component analysis (PCA) results of the RNA sequencing data. (B) Cluster gram heat map. These data demonstrated that there were significantly up-regulated/down-regulated genes in the host cells under different osmotic pressures.

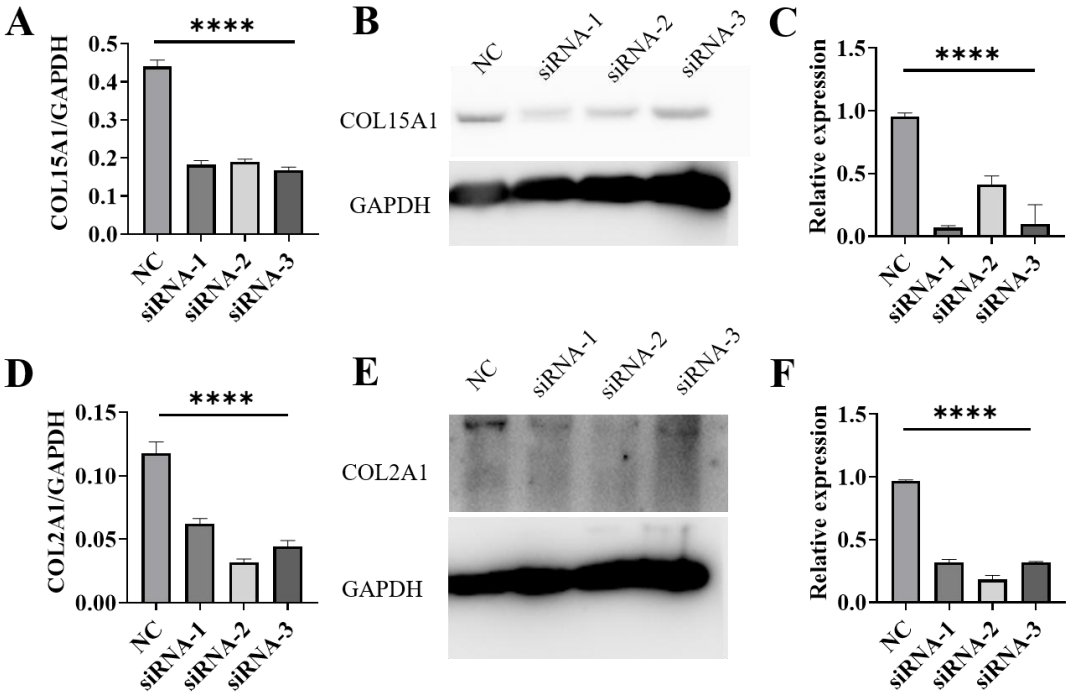

Fig. S8. Experiments on siRNA-mediated Knockdown of type XV and type II collagen in IEC-6 cells. (A) Relative expression of COL15A1 in the mRNA level. (B) Western blot (WB) and (C) the corresponding quantitative results for IEC-6 cells that were transfected with the designed siRNA sequences targeting COL15A1 or negative control (NC) siRNA. (D) Relative expression of COL2A1 in the mRNA level. (B) Western blot (WB) and (C) the corresponding quantitative results for IEC-6 cells that were transfected with the designed siRNA sequences targeting COL2A1 or negative control (NC) siRNA. All the statistical data were presented as Mean±SD from at least three independent experiments for each specific condition. Statistical analyses based upon one-way ANOVA were used in the experiments, and *, **, *** and **** indicated $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

Table S1. Preparation of osmotic stimulation solutions with different osmotic pressures.

Experimental group	Reagent formula	Measured osmotic pressure (mOsm łkg^{-1})
0.5 X	Regular DMEM Medium[a] + ddH_2O (v/v=1:1)	168
0.75 X	Regular DMEM Medium + ddH_2O (v/v=2:1)	227
1 X	Regular DMEM Medium	344
1.5 X	Regular DMEM Medium + 30mg/ml mannitol	499
2 X	Regular DMEM Medium + 50mg/ml mannitol	612

[a] DMEM/High glucose produced by Hyclone (Cat No.: SH30243.01) was referred to as isotonic solution (1 X) in the experiments.

Table S2. The number of significantly up-regulated and down-regulated genes in IEC-6 cells under hypertonic and hypotonic conditions

Sample comparison	Up-regulated	Down-regulated
Hypotonic vs. Isotonic	2399	667
Hypertonic vs. Isotonic	2113	566

Table S3. List of primer sequences designed for quantitative reverse transcription PCR.

Target Gene		**Sequence(5'—3')**
COL15A1	Forward primer	GCCCCCTACTTCATCCTCTC
	Reverse primer	CAGTACGGACCTCCAGGGTA
COL2A1	Forward primer	ACGCTCAAGTCGCTGAACAA
	Reverse primer	TCAATCCAGTAGTCTCCGCTCT
GAPDH	Forward primer	CCGCATCTTCTTGTGCAGTG
	Reverse primer	CGATACGGCCAAATCCGTTC

Table S4. List of siRNA sequences designed in the experiments.

Species	Target Gene	Name		Sequence(5'—3')
Rattus norvegicus	COL15A1	Si-1	sense	GCU CAU UGG UGU CCC AUU ATT
			antisense	UAA UGG GAC ACC AAU GAG CTT
		Si-2	sense	GGA AGU AGA CAU GCU GGA UTT
			antisense	AUC CAG CAU GUC UAC UUC CTT
		Si-3	sense	GCC UAA AGA AGC ACA CGU UTT
			antisense	AAC GUG UGC UUC UUU AGG CTT
	COL2A1	Si-1	sense	GCU GGU GCA CAA GGU CCU ATT
			antisense	GCU GGU GCA CAA GGU CCU ATT
		Si-2	sense	UAG GAC CUU GUG CAC CAG CTT
			antisense	GCU CAU CCA GGG CUC CAA UTT
		Si-3	sense	GGG UGA AGG UGG AAA GCA ATT
			antisense	UUG CUU UCC ACC UUC ACC CTT
	Negative control		sense	UUC UCC GAA CGU GUC ACG UTT
			antisense	ACG UGA CAC GUU CGG AGA ATT